DNA Microarrays for Characterization of Microbial Backgrounds

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RESEARCH OBJECTIVES

The primary goal of this project is to understand the quantity and composition of background microorganisms in the environment and to define and predict their ability to interfere with DNA-based pathogen detection systems. By providing baseline knowledge of bacterial organisms in urban aerosols and other environmental samples, this work will make it possible to predict the sensitivity, accuracy, and reliability of DNA-based detection schemes under "real world" conditions. Characterization of bioaerosols is also important for determining the long-term effects of introducing engineered microorganisms for biopesticides and bioremediation on downwind environments.

APPROACH

Sequence variation within the 16S rRNA gene was used to provide an effective method for the identification of bacteria in environmental samples without the need for their cultivation. Taking advantage of the fact that all bacteria possess a 16S rRNA gene, we developed a high-density oligonucleotide microarray for simultaneous identification of all bacterial components in any complex environmental sample. Multiple, sequence-specific probes target sections of the gene that are unique to each species. The combinatorial power of multiple probes increases the confidence of correct species identification. Our latest design has 500,000 probes arrayed on a 1.3 cm^2 surface. The unique discriminatory power of this microarray allows, for the first time, a high-throughput method for fine-scale microbial species composition measurements. Thus, it is possible to measure, over time, the fate of hundreds of different species in a complex microbial community subject to meteorological or other variations.

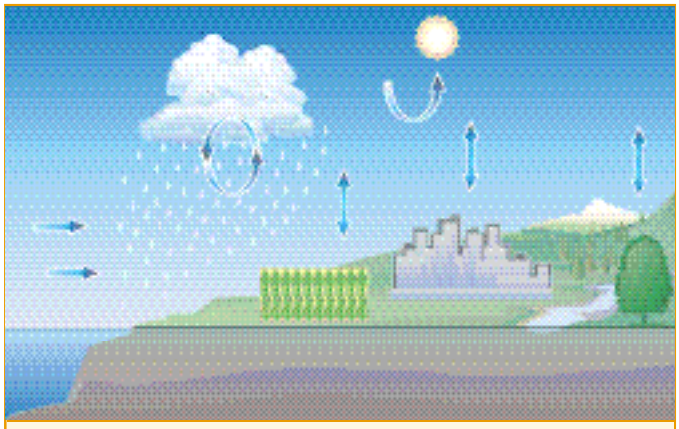

Figure 1. The microbial composition in the atmosphere is highly dynamic. Organisms are released into the air from both local and long-range sources.

ACCOMPLISHMENTS

We collected replicated aerosol samples from two biosurveillance studies targeting 12 U.S. cities and from an eight-site longitudinal transect comparing urban and rural bacterial community composition. Changes in microbial community composition were observed from city to city and from urban to rural areas. Information on bacterial species composition and relative amounts as determined by the strength of the hybridization interaction is being placed on a database. An information retrieval network is being established that will allow researchers to identify predominant organisms for specific cities, seasons, or other user-defined variables.

SIGNIFICANCE OF FINDINGS

Variations from site to site suggest that local reservoirs play a significant role in bacterial community composition. The increased diversity of urban sites over rural sites further strengthens this observation, with a greater number of distinctive habitats in the urban areas. The sequence-specific hybridization of 16S rRNA to a microarray allows the gathering of detailed information on microbial composition and diversity for any environmental sample. By comparing the microbial species composition before and after an environmental perturbation, key organisms may be identified.

RELATED PUBLICATIONS

Wilson, K. H., W.J. Wilson, , J.L. Radosevich, T. Z. DeSantis, V. S. Viswanathan, T.A. Kuczmarski, and G.L. Andersen, High density microarray of small subunit ribosomal DNA probes. Appl. Env. Micro., 68 (5), 2535–2541, 2002.

DeSantis, T. Z., I. Dubosarskiy, and G.L. Andersen, Comprehensive aligned sequence construction for automated design of effective probes (CASCADE-P) using 16S rDNA. Bioinformatics, 19 (July 2003 issue).

ACKNOWLEDGMENTS

This work was supported by the National Nuclear Security Administration (NNSA) Chemical and Biological Non-Proliferation Program, NN-22, for the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.